Proc. Natl. Acad. Sci. USA
Vol. 76, No. 6, pp. 2932–2936, June 1979
Immunology

Amino acid sequence of a mouse immunoglobulin μ chain

(automated sequencer/immunoglobulin domain/myeloma tumor MOPC 104E/immunoglobulin evolution/carbohydrate attachment sites)

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Communicated by Edward B. Lewis, March 26, 1979

ABSTRACT **The complete amino acid sequence of the mouse μ chain from the BALB/c myeloma tumor MOPC 104E is reported. The C_μ region contains four consecutive homology regions of approximately 110 residues and a COOH-terminal region of 19 residues. A comparison of this μ chain from mouse with a complete μ sequence from human (Ou) and a partial μ chain sequence from dog (Moo) reveals a striking gradient of increasing homology from the NH_2-terminal to the COOH-terminal portion of these μ chains, with the former being the least and the latter the most highly conserved. Four of the five sites of carbohydrate attachment appear to be at identical residue positions when the constant regions of the mouse and human μ chains are compared. The μ chain of MOPC 104E has a carbohydrate moiety attached in the second hypervariable region. This is particularly interesting in view of the fact that MOPC 104E binds α-(1→3)-dextran, a simple carbohydrate. The structural and functional constraints imposed by these comparative sequence analyses are discussed.**

Immunoglobulins are comprised of two polypeptides, light (L) and heavy (H) chains, and may be divided into five major classes, IgM, IgG, IgA, IgD, and IgE, which are defined by their corresponding heavy chains, μ, γ, α, δ, and ε, respectively. The IgM molecule is the most primitive immunoglobulin in that it is the first to appear in vertebrate evolution (1). Moreover, IgM is the first immunoglobulin to appear during the ontogenetic development of the immune system (2). Serological, immunochemical, and evolutionary data suggest that IgM immunoglobulins have been more highly conserved than other classes of immunoglobulins (1). For these reasons, the IgM molecule is an excellent model protein for evolutionary analysis.

Each immunoglobulin chain is divided into an NH_2-terminal variable (V) region and a COOH-terminal constant (C) region. The immunoglobulin polypeptides are divided into consecutive homology regions approximately 110 residues in length. The L chain has two homology regions and the human μ chain has five homology regions in addition to a COOH-terminal segment composed of 19 residues (3). The basic unit of the immunoglobulin molecule is two light and two heavy chains (H_2L_2), in which pairs of homology regions fold together to form globular domains. The L chain and the NH_2-terminal portion of the H chain generate two domains each, and the remaining COOH-terminal regions of the two μ chains fold into three domains. From the NH_2 to the COOH terminus of the μ chain, these domains are designated V, $C_\mu1$, $C_\mu2$, $C_\mu3$, and $C_\mu4$, respectively.

The V domains recognize foreign structures (antigens) and the C domains carry out a variety of effector functions that ultimately lead to the destruction or elimination of foreign antigens. The IgM molecule has at least three special effector functions associated with its COOH-terminal domains. First, the IgM molecule serves as a cell-surface receptor that can trigger the progenitors of antibody-producing B cells to differentiate upon interaction with complementary antigen (4). Thus the IgM molecule can be an integral membrane protein (5, 6), which is displayed on the membrane as a 7S monomer, (μ_2L_2) (7, 8). Second, cells that have been stimulated to differentiate into antibody-producing cells can secrete IgM molecules as 19S pentamers, $(\mu_2L_2)_5$, composed of 10 light and 10 heavy chains held together by a third type of polypeptide designated the J chain (9). It is not known whether the membrane-bound and secreted μ chains have the same polypeptide structure. Finally, after the IgM molecule binds antigen, the $C_\mu4$ domain activates the effector pathways of the complement system (10).

In this paper we report the amino acid sequence of the secreted μ chain derived from the mouse myeloma tumor MOPC 104E. The mouse μ chain is compared with a human μ chain whose sequence has been completely determined (11) and a dog μ chain whose sequence has been partially determined (12, 13). As previously observed when the human μ sequence and the dog μ partial sequence were compared, there is a striking gradient of sequence homology, with the COOH-terminal homology regions significantly more conserved than the NH_2-terminal homology regions (12). Several structural features of the μ chain are highly conserved, including the placement of disulfide bridges and the locations of tryptophan residues and carbohydrate moieties in the constant region. The structure of the secreted μ chain places several interesting constraints on the nature of the membrane-bound μ chain of the IgM receptor molecule.

MATERIALS AND METHODS

Isolation of μ Chain. The IgM-secreting tumor MOPC 104E (μ, λ) was grown intraperitoneally in (BALB/c × DBA/2) F_1 mice. The IgM molecules were precipitated from ascites fluid with ammonium sulfate and purified by gel filtration on a column of ACA 22 (LKB). The purified IgM was completely reduced with dithiothreitol and alkylated with iodoacetamide in 6 M guanidine·HCl (14), and the heavy and light chains were separated by gel filtration on a column of ACA 34 (LKB) in 3 M guanidine and 0.2 M ammonium bicarbonate. The complete amino acid sequence of the λ light chain of MOPC 104E has been determined (15, 16).

Isolation of Cyanogen Bromide Fragments. Purified μ chains were cleaved with cyanogen bromide in 70% (wt/vol) formic acid for 20–22 hr at 4°C (17). Cyanogen bromide peptides were separated by gel filtration on a column of ACA 54 (LKB) in 3 M guanidine and 0.2 M ammonium bicarbonate.

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Abbreviations: H and L, heavy and light chains of immunoglobulins; V and C, variable and constant regions of immunoglobulin chains; CHO, carbohydrate.

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Protein and peptide purity was monitored at all stages of purification by electrophoresis in the presence of sodium dodecyl sulfate on 10% or 18% polyacrylamide gels (18).

Compositional and Sequence Analysis. Amino acid compositions and carbohydrate analyses for amino sugars were determined on samples hydrolyzed in 6 M HCl (110°C for 24 hr and 3 hr, respectively) by using a Durrum D-500 amino acid analyzer. Automated sequence analyses were performed on a modified Beckman sequenator (19, 20). Samples were loaded by using Polybrene (Aldrich), and phenylthiohydantoin derivatives were identified by high-pressure liquid chromatography (Waters Associates) as described (21).

Peptide Fragments. Fc fragments of IgM were prepared according to the method of Shimizu *et al.* (22) and purified by gel filtration on ACA 22 and ACA 34. Cyanogen bromide peptides were succinylated (23) for trypsin digestion limited to arginine residues. Cyanogen bromide peptides also were cleaved at tryptophan residues by the procedure of Ozols *et al.* (24). Smaller peptides were produced from cyanogen bromide fragments by digestion with trypsin, chymotrypsin, and thermolysin. The resulting peptides were separated in two dimensions on paper by chromatography and high-voltage electrophoresis (25) and where appropriate their amino acid sequences were determined in the automated sequenator in the presence of Polybrene (see ref. 21).

RESULTS

Sequence Strategy. The methionine residues of the MOPC 104E μ chain were cleaved with cyanogen bromide and 10 peptides were isolated by gel filtration. The amino acid sequences of these peptides were determined at their NH_2 termini on the automatic sequenator. The sequences of selected peptides produced by tryptophan, thermolysin, trypsin, and chymotrypsin cleavages also were determined to complete the primary structure of each cyanogen bromide fragment. Fig. 1 schematically illustrates the order of the cyanogen bromide fragments. Only fragment CN1 was not isolated. Two cyanogen bromide fragments that resulted from incomplete cleavage of the methionine residues at positions 20 (CN1-2) and 568 (CN8-9) were isolated. Thus, cyanogen bromide fragments covering the entire μ chain have been obtained and their sequences have been completely determined.

The 10 methionine fragments of the mouse μ chain can be unambiguously aligned in a linear order without resorting to homology comparisons with the human μ chain. Sequence analysis of the NH_2-terminal 38 residues of the intact μ chain assigned cyanogen bromide fragments 1, 2, and 3, respectively, to the NH_2 terminus (Fig. 1). An Fc fragment, obtained by trypsin digestion of the intact IgM in 5 M urea (22), shows that the COOH-terminal sequence of CN5 and the NH_2-terminal sequence of CN6 are contiguous (Fig. 1). In addition, a cyanogen bromide digest of the Fc fragment yielded CN6, CN7, and CN8-9. Because CN4 is not in the Fc fragment, it must reside between CN3 and CN5 (Fig. 1). Because CN8-9 does not have a COOH-terminal homoserine residue it must be the COOH-terminal fragment. The only remaining fragment, CN7, is then located between CN6 and CN8-9 (Fig. 1). These assignments are supported by the previous isolation and sequence analysis of pepsin peptide fragments overlapping CN4 to CN5 and CN7 to CN8 (27). Thus direct sequence overlaps are available for all methionine residues except those between CN6-CN7 and CN3-CN4. There is the possibility that additional methionine fragments between CN3-CN4 and CN6-CN7 may have been lost in the isolation procedures. This possibility appears unlikely because the mouse μ chain in these two regions can be aligned without sequence gaps against a completely sequenced human μ chain (Fig. 2).

Disulfide Bridges. The positions of the 14 cysteine residues of the mouse μ chain are indicated in Fig. 1. The assignments of two disulfide bridges (residues 22 to 97; residues 153 to 213) have been established through the isolation of cyanogen bromide peptides from μ chain with intact intrachain bridges followed by analysis on reducing and nonreducing 18% polyacrylamide gels. In addition, the methionine fragments of the Fc region, CN6, CN7, and CN8, are linked by intrachain disulfide bridges. All 14 cysteine residues in the mouse μ chain

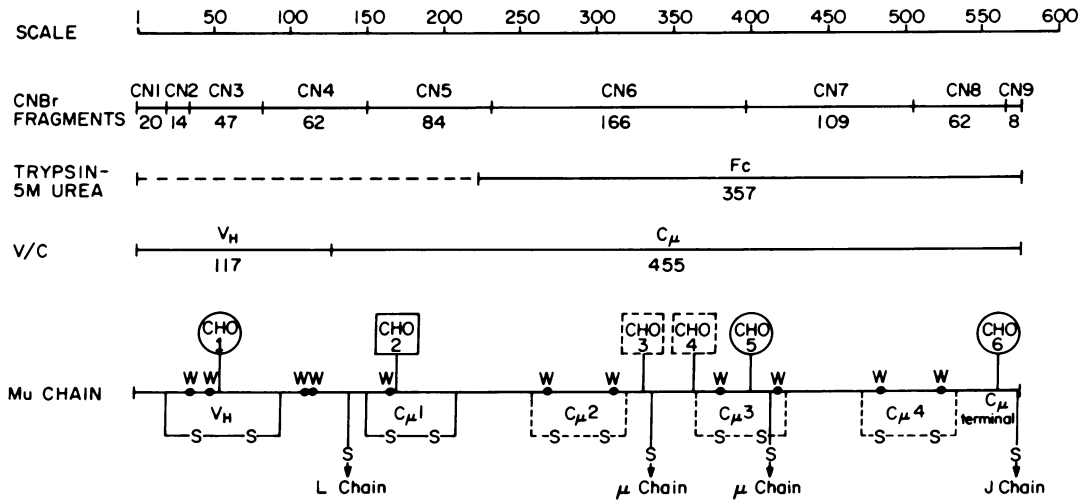

FIG. 1. Schematic drawing of the μ heavy chain of IgM MOPC 104E showing (*i*) sites of cleavage by CNBr and the respective fragments (CN1 to CN9), (*ii*) the point of cleavage by trypsin in 5 M urea giving the Fc fragment, (*iii*) the point of division of the variable (V_H) and constant (C_μ) regions (3), (*iv*) the intrachain disulfide bridges, (*v*) the six homology regions (V_H, $C_\mu 1$ to $C_\mu 4$, C_μ terminal), and (*vi*) the locations of the six oligosaccharides (CHO 1 to CHO 6). The high-mannose oligosaccharides are circled and complex type oligosaccharides are boxed. Two oligosaccharides are enclosed in broken lines to denote that the assignment of complex type was made from homology with the human μ oligosaccharides (26). The linkages of three of the intrachain bridges are indicated by broken lines because they have been assigned only by assuming homology with the human μ sequence Ou (11). W indicates tryptophan residues. To facilitate homology comparisons, the scale corresponds to the amino acid sequence positions of the human myeloma μ chain Ou (11). Because there are a number of gaps and insertions between the two sequences, the numbers indicating total amino acid residues in each 104E μ chain fragment do not necessarily correspond to the numbers obtained by counting the amino acid residues in the homologous Ou sequence.

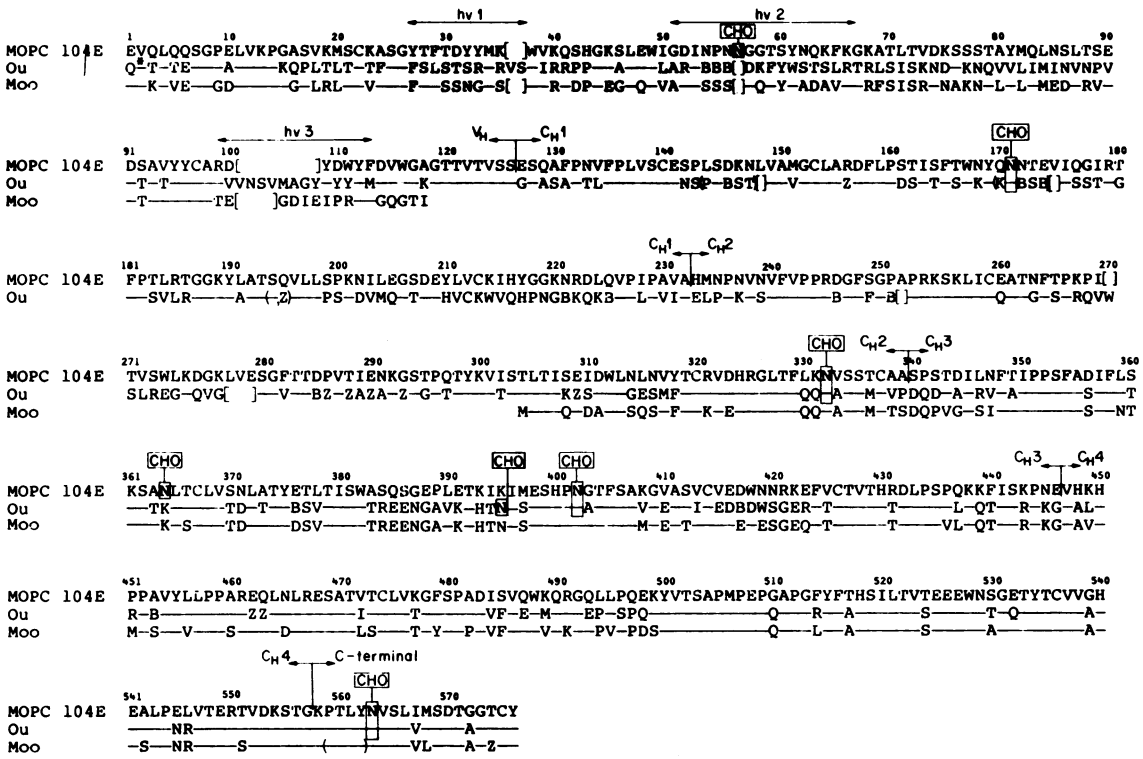

FIG. 2. The amino acid sequences of μ chains from mouse MOPC 104E, human Ou (11), and canine Moo (12, 13). (The one-letter code for amino acids is given in ref. 28.) Only the NH_2-terminal and Fc sequences of the dog μ chain are available. A straight line indicates identity with the MOPC 104E sequence. Deletions are indicated by [ ]. The sites of carbohydrate attachment are boxed. Positions of carbohydrate attachment have not been determined for the dog μ chain. The homology unit boundaries have been assigned by an analysis of immunoglobulin three-dimensional structure as indicated in Table 1 (3). Three hypervariable regions (hv 1, hv 2, and hv 3) are indicated by arrows over appropriate V region segments. Q* indicates pyrrolidone carboxylic acid.

are in positions homologous to the 14 cysteine residues of the human μ chain. Thus we assume the same cysteine residues will be involved in interchain bridges and intradomain bridges as indicated in Fig. 1. Accordingly, the cysteine residue at position 140 is probably bridged to the L chain and the cysteines at 337, 414, and 575 most likely form bridges with other μ chains or with the J chain (16, 29).

Oligosaccharide Assignments. The 104E μ chain has six sites of carbohydrate attachment, five in the constant region and one in the variable region (Fig. 1). All six oligosaccharides contain glucosamine and are attached to asparagine residues. The sites of carbohydrate attachment were identified as blank cycles in automatic sequenator runs. Subsequent enzymatic limit digests of the carbohydrate-containing cyanogen bromide fragments produced small peptides that were analyzed for the presence of glucosamine. Four of the five C region oligosaccharides of the mouse μ chain are in positions identical to those of the human μ chain (Figs. 1 and 2). The fifth C region oligosaccharide is attached to a nonhomologous position 31 residues NH_2-terminal to the site in Ou (positions 395 and 364 in human and mouse, respectively).

DISCUSSION

The COOH-Terminal Portion of the C_μ Region Appears More Highly Conserved Than the NH_2-Terminal Portion. In Fig. 2 the complete sequence of the μ chains of mouse and human are listed along with the V region and Fc sequence data from the dog μ chain. Several insertions, deletions, and sequence inversions, some of which may be technical artifacts in analysis (28), are noted in comparing these sequences. Using the criteria of Beale and Feinstein (3), we have identified in the mouse C_μ, as in its human counterpart, four homology regions of approximately 110 residues and an additional COOH-terminal region 19 residues in length (Fig. 2). The mouse μ chain, like the human μ chain, has no hinge region separating any of the C region domains (3).

Two amino acid residues, cysteine and tryptophan, are highly conserved in the C_μ regions (Figs. 1 and 2). All 12 cysteine residues are conserved in the mouse and human C_μ regions, as are the 8 that can be compared in the dog sequence. The mouse C_μ region has six tryptophan residues, each of which is homologous to one of the seven tryptophan residues in the human C_μ region (Fig. 2). All five tryptophans in the partial sequence of the dog C_μ region are in the same positions as their mouse and human counterparts. In contrast, the human and mouse C_μ regions have six and five methionine residues, respectively, only two of which are conserved. Because of their conservation, it is reasonable to conclude that cysteine and tryptophan residues play an important role in the domain structure of immunoglobulins.

Table 1 is a compilation of the homology comparisons between the various homology regions of mouse, human, and dog. There is a striking gradient of homology from the NH_2-terminal to the COOH-terminal region. The $C_\mu1$ homology unit of mouse shows 48% homology with human μ as contrasted with 78% and 89% homologies in the $C_\mu4$ and the COOH-terminal regions, respectively. The existence of sequence gaps in the NH_2-terminal homology regions (two in $C_\mu1$ and three in $C_\mu2$) but not in the COOH-terminal homology regions of the μ chain (Fig. 2) suggests that the NH_2-terminal regions have diverged more. Thus, the COOH-terminal portion of the μ chain is significantly more conserved than the NH_2-terminal portion. If individual domains do carry out separate functions, these observations imply that there are strong selective constraints on

Table 1. Homology matrix of the constant region domains of mouse, human, and dog μ chains

Comparison	V	C_H1	C_H2	C_H3	C_H4	C-Terminus
Mouse 104E vs. Human Ou	38% / 116/116	48% / 106/106	59% / 106/106	53% / 107/107	78% / 111/111	89% / 19/19
Dog Moo vs. Human Ou	38% / 112/112		69% / 36/106	79% / 107/107	79% / 111/111	89% / 19/19
Mouse 104E vs. Dog Moo	44% / 108/108		56% / 36/106	56% / 107/107	72% / 111/111	79% / 19/19

The μ chains were divided into four homology units and a COOH-terminal region according to an analysis of the three-dimensional structures of several immunoglobulins not of the μ class: C_H1 = residues 127–232; C_H2 = residues 233–339; C_H3 = residues 340–446; C_H4 = residues 447–557; and C terminus = residues 558–576 (3). Sequence gaps (deletions or insertions) are not counted in homology comparisons. In the lower right of the square for each region and comparison is listed the number of amino acids compared in the homology calculation divided by the total number of amino acids in that homology unit. The number of sequence identities in each unit was used to calculate the percent homology, which is given in the upper left of each square.

the more COOH-terminal domains. These constraints may be related to the requirements for the polymerization of pentamers in the secreted IgM molecule, the structural requirements imposed on an integral membrane receptor with regard to membrane attachment and the triggering of differentiation, or the activation of the complement pathway, which has been localized to the$C_\mu4$ domain (10).

Sites of Carbohydrate Attachment to the C_μ Region Are Similar in Human and Mouse μ Chains. The μ chain of mouse has five sites of carbohydrate attachment in the constant region (Fig. 1). Four of these five carbohydrate attachment sites are at identical positions in the mouse and human (26) C_μ regions (Fig. 2). The carbohydrate moiety on residue 171 is of the complex type and those on residues 402 and 563 are of the high-mannose type (W. J. Grimes, personal communication), in agreement with the carbohydrate types on the human μ chain (26). Compositional analyses on the remaining two oligosaccharides are not yet complete. Each carbohydrate moiety is attached to the asparagine residue of a three-residue recognition sequence, -Asn-X-Ser/Thr, in which X may be any amino acid and Ser/Thr indicates either serine or threonine. Substitution of a methionine for a serine at position 397 in mouse μ has eliminated the recognition sequence for the carbohydrate attachment site at position 395 in human μ. In the mouse μ chain we find a carbohydrate moiety at residue 364, where there exists a carbohydrate recognition sequence that is absent in the dog and human μ chains. Other recognition sequences that are apparently not glycosylated also occur in the μ chain (74–76 in human Ou, 263–265 and 347–349 in mouse MOPC 104E). Thus, the elimination and formation of an attachment site implies either that this region of the protein is on the exterior of the molecule and is therefore accessible to glycosylating enzymes or that a carbohydrate moiety is required (structurally, functionally, or both) in this region of the μ chain.

The carbohydrate moieties of human and mouse μ chains are present in the $C_\mu1$, $C_\mu2$, and $C_\mu3$ domains as well as the COOH-terminal region (Figs. 1 and 2). A variety of functions have been suggested for the carbohydrate on heavy chains, including the solubilization of the heavy chain, the facilitation of the secretory process, the promotion of the assembly of pentamers, and the contribution of structural features for a variety of specific effector functions (26, 30–34). Whatever their function, the conservation of the locations of carbohydrate attachment during the 75 million years that mouse and human C_μ genes have diverged from one another suggests that strong selective forces are maintaining the recognition sequences.

The V Domain of MOPC 104E, an α-(1→3)-Dextran Binder, Has a Carbohydrate Moiety in Its Antigen-Binding Site. A carbohydrate moiety has been identified at asparagine 57, which lies in the middle of the second hypervariable region of the MOPC 104E μ chain. There are several unusual features of the V region carbohydrate site. First, the usual recognition sequence is missing; this high-mannose carbohydrate moiety is attached to the asparagine of an Asn-Gly-Gly-Thr sequence. Although the majority of asparagine-linked carbohydrate moieties are associated with a recognition sequence, the sequence is clearly not an absolute requirement for the attachment of carbohydrate to asparagine residues (35). Second, the IgM molecule secreted by the MOPC 104E tumor binds a simple carbohydrate, α-(1→3)-dextran (36). Thus, we have localized a carbohydrate moiety to the antigen-binding site of the heavy chain of an immunoglobulin that binds a defined hapten. This observation raises the possibility that carbohydrate–carbohydrate interactions may constitute a portion of the binding energy for the hapten, α-(1→3)-dextran. In view of what we know about the specificities of protein and carbohydrate interactions, however, this possibility appears unlikely (37). Third, a second myeloma protein, J558(α, λ), which also binds α-(1→3)-dextran, appears to have a carbohydrate moiety at precisely the same position (unpublished observations). It is likely that this carbohydrate moiety also occurs in the heavy chains of normally induced serum antibodies to α-(1→3)-dextran (unpublished observations). The role, if any, for carbohydrate in the V domains of immunoglobulins remains an interesting, but unanswered question.

Structure of the μ Chain Places Constraints on the IgM Molecule as an Integral Membrane Receptor. The monomeric IgM molecule is an integral membrane protein that serves as a cell-surface receptor for antigen on B cells that can trigger the B cells to differentiate into antibody-producing cells (4). The membrane (m) and secreted (s) IgM molecules are serologically crossreactive, implying that they share structural features or may even be identical. Conflicting data suggest both that the COOH terminal portion of the μ_m chain is identical to μ_s (38) and that these regions are different (39).

If the μ_m and μ_s chains are identical in amino acid sequence, the μ_m chain cannot be transmembrane in its orientation. The α-helix is the most stable polypeptide secondary structure within a membrane (40, 41). This configuration requires about 21 uncharged residues to span the membrane. Alternatively, the extended β configuration requires two paired stretches of 9 uncharged amino acids to span the membrane. We have compared the three mammalian μ chains over their COOH-terminal two homology units, which presumably contain the site of membrane attachment. The longest sequence of uncharged amino acids in any of these molecules is 14 residues in length (positions 469–482 in the human and dog μ chains) (Fig. 2). However, the mouse μ chain has a charged lysine residue at position 477, reducing the stretch of uncharged amino acids in this region to 8 residues. In addition, this region contains a cysteine at position 474 that is presumably joined to cysteine-536 to form the $C_\mu 4$ intradomain disulfide bridge. If one assumes that all mammals will attach their μ chains to membranes in a similar fashion, there is no uncharged sequence shared by the COOH-terminal regions of the mouse, human, or dog μ chains that could span the membrane in either the α-helical or the extended unpaired β configuration (41, 42). We conclude that, if the μ_m and μ_s chains are identical, the IgM molecule cannot be a transmembrane protein that meets the above specifications. Moreover, tyrosine-labeling experiments on inside-out vesicles are consistent with the supposition that the IgM molecule is not transmembrane (43). If the μ_m chain is not a transmembrane polypeptide, then the IgM molecule may associate with the membrane by virtue of a specific receptor molecule, an association analogous to the interaction of IgE with its membrane receptor on mast cells (44). In this regard, both IgM and IgE have an additional constant region domain when compared with IgG and IgA molecules (3).

We have preliminary evidence that suggests the μ_m and μ_s chains differ in their primary amino acid sequences. Any primary structural differences could be generated by mRNA splicing mechanisms, posttranslational proteolysis, or the occurrence of duplicated μ chains genes. It is possible to determine the chemical structure of μ_m and define precisely the relationship between μ_m and μ_s chains. Moreover, we have isolated a genomic μ clone, whose nucleotide sequence can be determined. Such studies should allow us to distinguish unambiguously among the three models for μ_m and μ_s expression cited above.

We thank V. Farnsworth and M. W. Hunkapiller for patiently teaching techniques of peptide and amino acid sequence analyses. This work has been supported by National Institutes of Health Grants AI-10781 and CA-20314 and by a Gordon Ross Medical Foundation Fellowship to M.K.

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